THE EFFECT OF INTRAUTERINE GROWTH RESTRICTION ON THE EXPRESSION OF THE PPARγ TARGET GENE, SETD8 IN ADIPOSE AND LIVER OF THE RAT

by

Ornela Bashaj Bailey

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Division of Nutrition

The University of Utah

August 2011

Copyright © Ornela Bashaj Bailey 2011

All Rights Reserved

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Ornela Bashaj Bailey**

has been approved by the following supervisory committee members:

Eldon W. Askew	, Chair	**05/26/11** Date Approved
Lisa Anne Joss-Moore	, Member	**05/26/11** Date Approved
Thunder Jalili	, Member	**05/26/11** Date Approved

and by **Eldon W. Askew** , Chair of

the Department of **Division of Nutrition**

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

Intrauterine growth restricted (IUGR) born individuals are at a higher risk for developing obesity and associated co-morbidities in adulthood. An underlying component of these morbidities is adipose dysfunction, with the accumulation of visceral adipose tissue (VAT) in conjunction with an increased expression of peroxisome proliferator activated receptor gamma PPARγ, a proadipogenic factor. Interestingly, in hepatic tissue of the adolescent IUGR female, PPARγ expression displays a decreasing trend. PPARγ's actions may be mediated by set domain containing histone methyltransferase Setd8 an epigenetic modification enzyme, and direct transcriptional target of PPARγ. In this study, we hypothesize that IUGR will affect mRNA and protein levels of Setd8 in a gender and tissue specific manner in visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and hepatic depots before the onset of metabolic disease in postnatal day 21 rats. Uteroplacental insufficiency (UPI) induced IUGR day 21 adipose (SAT and VAT), and hepatic tissue was compared to control tissue using real time RT PCR for mRNA and western blotting for protein levels of Setd8. IUGR increased Setd8 protein levels in visceral adipose tissue of males of and decreased Setd8 protein levels hepatic tissue of females. IUGR did not have a significant effect on SAT mRNA and protein levels. This study demonstrates that IUGR alters Setd8 expression in day 21 rat in a gender and tissue specific manner, consistent with previously observed changes in PPARγ expression, before the onset of metabolic disease and obesity. We

speculate that IUGR alters epigenetic gene regulation in adipose and liver, PPARγ and its target enzyme Setd8, and that this may contribute to adult onset obesity observed in this population.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

ACKNOWLEGMENTS

I would like to thank committee members Lisa Joss-More, Wayne Askew and Thunder Jalili for their direction, assistance and support with this project. I would also like to gratefully acknowledge all members of the Lane Lab, especially Yan Wang for her patience, assistance and guidance while I was learning new research techniques in the lab. This project would not have been possible without Lisa Joss-Moore's continued support, encouragement and unwavering guidance. I could not imagine having a better mentor. I would like to extend my deepest gratitude to my husband Thomas Bailey for all his love, emotional support and patience during the past 2 years. Finally, I would like to thank my parents Hekuran Bashaj and Fiqerete Bashaj for selflessly supporting me, believing in me and unconditionally loving me. This thesis is dedicated to them.

INTRODUCTION

Intrauterine Growth Restriction

Intrauterine growth restriction (IUGR) affects 5-12% of infants in developed nations every year, (1) and is mainly caused by uteroplacental insufficiency (UPI) (2). Preeclampsia is the leading cause of UPI induced IUGR in humans (3). UPI refers to the reduced amount of blood flow from the uterus to the placenta, resulting in decreased nutrients and oxygen to the fetus. Given these conditions, the fetus fails to achieve its genetic growth potential (4, 5). During critical periods of prenatal development, poor nutrition and other environmental factors induce epigenetic variation and gene expression changes leading to permanent changes in metabolism and chronic disease susceptibility (6). Evidence in human and animal studies (7) shows that infants who are born IUGR are at a higher risk for heart disease, hypertension, type 2 diabetes, abnormal glucose-insulin metabolism and increased serum cholesterol levels (6). IUGR infants have physiologically impaired adipose tissue regulation, which is accompanied by adult onset metabolic disease, insulin resistance, poor fatty acid utilization, hepatic steatosis and obesity in a gender specific manner (8).

Adipocyte Dysfunction Linked to Metabolic Disease

In addition to its ability to store triglycerides adipose tissue is a complex, active endocrine organ, capable of sending and receiving signals in order to modulate energy

expenditure, reproduction, and sensitivity to insulin (9). Interestingly, IUGR adipose tissue regulation seems to be physiologically dysregulated before the onset of obesity and metabolic disease (10). For example, IUGR infants are born smaller, buy they experience accelerated weight gain and obtain more adipose tissue during childhood when compared to infants who achieve full genetic growth potential at birth (2, 11). In addition, IUGR favors the accumulation of Visceral Adipose Tissue (VAT) rather that Subcutaneous Adipose Tissue (SAT) in a gender specific manner. MRI scans of IUGR male rats show an increased amount of VAT and VAT per gram body weight on postnatal day 21, indicating increased lipid storage (12). VAT and SAT have different functions and this differences in distribution and depots effect gene regulation and ultimately the phenotype. The increase amount of VAT is metabolically detrimental. This shift towards VAT is consistent with adult onset metabolic disease and altered adipose function. Further, increased VAT has been reported in obesity and high fat diet models (13) in male animals (14).

PPARγ

The increase in VAT in male IUGR rats is associated with an increased expression of peroxisome proliferator activated receptor gamma (PPARγ). PPARγ is part of the PPAR nuclear receptor super family, and is a transcription factor that binds to and alters the transcription of a number of genes involved in cellular metabolism, adipogenesis, and lipid storage (15). The PPARγ gene translates two protein isoforms: PPARγ1, which is the widely expressed including in the liver, and PPARγ2, which is specific to adipose, liver and lung tissues. This distinct tissue specificity is attained via alternative splicing, and promoter usage (16). PPARγ2 transcriptional activity is ligand

dependent. Docosahexanoic acid (DHA), fatty acid derivatives and the insulin sensitizing drugs are PPARγ2 ligands. They cause a conformational change affecting transcription of PPARγ2 and its target genes, ultimately improving insulin sensitivity. PPARγ2 isoform is a vital regulator of adipocyte differentiation, maintenance, and survival (17). In rat IUGR models where IUGR is induced by either 50% maternal food restriction during gestation or UPI, it has been shown that PPARγ2 is up-regulated in VAT of male IUGR rats (12, 18). In preliminary studies, IUGR significantly increased PPARγ2 mRNA expression in liver of day 21 male rats, promoting lipid deposition and storage and ultimately resulting in increased hepatic steatosis. Interestingly, PPARγ1 mRNA in the liver is decreased at postnatal day 0, and postnatal day 21 in IUGR female animals.

A further role of PPARγ involves the transcriptional regulation of epigenetic modifying enzymes. Epigenetics refers to heritable changes in gene expression potential that are not caused by changes in DNA. A mechanism of epigenetic gene regulation is histone modifications. Chromatin is made up of DNA associated with histone proteins. Chromatin conformation is regulated differently in different tissues by number of modifications to residues in the tails of histone proteins. Epigenetic modifying enzymes place epigenetic markers on the residues of histone tail proteins that are associated with a particular gene. Some of these markers include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation (19). The mark dictates the interactions of the gene with all the transcription factors. Setd8, set domain containing histone methyltransferase, has a PPAR response element in its promoter region and it is a bona fide transcription factor of PPARγ. Several lines of evidence indicate that Setd8 is 352

amino acids long and its molecular mass is calculated at about 45 kDa (20). Although the underlying mechanism of activity of this methyltransferase remains unknown, its specific role in monomethylating histone H4 Lysine 20 (H4K20me1) is well documented (21). In adipose tissue, during differentiation PPARγ up regulates the expression of Setd8, which in turn increases transcription of the PPARγ gene and its targets such as fatty acid binding protein 4 (FABP4) and cluster of differentiation 36 (CD36) through H4K20me1 (15). Further, PPARγ binds to the PPARy2 promoter, inducing histone modification and ultimately increasing its transcription. This proposed mechanism links PPARγ and Setd8 transcription with epigenetic chromatin modulation through H4K20me1 during adipogenesis through a feedback loop (15).

Despite the known connection of IUGR and PPARγ expression in a gender and tissue specific manner, the effect of IUGR Setd8 expression in adipose and hepatic tissue is unknown. Therefore the purpose of this study is to assess the effect of IUGR on the PPARγ target gene Setd8 in the adipose and hepatic tissue of the rat. **We hypothesize that IUGR will affect mRNA and protein levels of Setd8 in a gender and depot specific manner in VAT, SAT and hepatic tissue reflecting the previously observed changes in PPARγ before the onset of metabolic disease in postnatal day 21 rats.**

MATERIALS AND METHODS

Model

All procedures were approved by the University of Utah Animal Care Committee are in accordance with the American Physiological Society's guiding principles (22). A more detailed procedure has been described previously (23). To briefly describe, on day 19 of gestation (term is 21.5 days), the maternal Sprague-Dawley rats were anesthetized with intraperitoneal xylazine (8 mg/kg) and ketamine (40 mg/kg), and both inferior uterine arteries were ligated (IUGR). Control dams underwent identical anesthetic procedures. The blood flow to the fetus was not ablated, but reduced to a similar degree as that is observed in human pregnancies complicated by UPI (8). The Lane Lab has extensive experience with the IUGR model, and has characterized the IUGR phenotype and gained mechanistic insights into the molecular and cellular response to the IUGR insult. Bilateral uterine artery ligation at gestational age 19 days (term is 21.5 days) results in asymmetric growth restricted pups that are approximately 25% smaller than the control animals at birth as well as at postnatal day 21 (24).

Maternal rats had ad libitum access to food and water and were allowed to deliver spontaneously at term approximately 2.5 days after the bilatereal uterine ligation. Litters were culled to 6 pups, 3 male and 3 female. The pups remained with their mothers, feeding via lactation, until day 21 of postnatal life. Juvenile and adult rats underwent euthanasia using a sodium pentobarbital overdose (150 mg/kg). Adipose tissue from

subcutaneous and retroperitoneal (visceral) depots was immediately harvested and flash frozen in liquid nitrogen and stored in -80°C. The same procedure was used for liver tissue. For each experiment, each group (control and IUGR) had 6 male pups and 6 female pups, unless otherwise noted. Pups within each group were derived from different litters.

Real-Time RT PCR

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to evaluate mRNA abundance in visceral, subcutaneous and liver tissues of Setd8 with GAPDH as an internal control. Total mRNA for IUGR and control was extracted from tissue crushed in liquid nitrogen and frozen at -80°C. RNeasy Lipid Tissue Kit (Qiagen, DB Bioscences CA) was used according to manufactures instruction. mRNA was quantified using a Nano Drop 3300 Flourospectometer and cDNA was synthesized using a High Capacity cDNA reverse Transcription Kit (Applied Biosystems, Foster CA). The following Assay-on-demand primer/probe set was used: Setd8 - Rn01477383_g1 (Applied Biosystems, Foster CA). GAPDH primer and probe sequences; Forward: CAAGATGGTGAAGGTCGGTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG. mRNA abundance was determined using the comparative Ct method with GAPDH as an internal control. All real-time PCR amplification, data acquisition and analysis were done using the 7900HT Real-time PCR system and SDS Enterprise Software (Applied Biosystems) using a 384-Well Optical Reaction Plate (Applied Biosystems) at the University of Utah genomics core facility. Taqman Universal PCR Mastermix (Applied Biosystems) was used in a 5μL reaction, performed in quadruplicate. Cycle parameters were 50°C x 2 minutes, 95°C x 10

minutes, followed by 40 cycles of 95°C x 15 seconds and 60°C x 60 seconds.

Protein Analysis

Western blotting was used to determine relative abundance of Setd8 proteins in SAT, VAT and liver tissue of IUGR compared to the control rats. Day 21 adipose tissue was crushed in liquid nitrogen using liquid nitrogen cooled mortar and pestle. RIPA buffer solution containing EDTA-free protease inhibitor tablets (Roch, Mannheim, Germany) was used to lyse the cells and extract total protein. The samples were vortexed thoroughly and placed on ice for 10 minutes. They were rotated for 60 minutes and centrifuged for 20 minutes at 4°C. Protein solution was isolated and stored in -20°C. A bicinchoninic acid protein assay (BCA) kit (Pierce, Rockford, IL) utilizing a 96-well plate was used to quantify the protein sample solutions. Identical procedures were followed for liver tissue with the following exceptions: 30-minute incubation time on ice in place of 10 minutes and a Fisher homogenizer was used to homogenize each sample for 30 seconds instead of using a rotator for 60 minutes.

Standard western blotting procedures were used with the following specifics. For adipose tissue, gel samples containing equal amounts of total protein (80μg) were prepared from the isolated adipose protein solution and run on a 4-12% bis-tris XT Criterion gels (Bio-Rad Laboratories, Hercules, CA) in MOPS buffer at 150V for 60 minutes. Proteins were transferred to PVDF membranes in transfer buffer at 100V for 60 minutes. Membranes were blocked in 5% milk TBS-T for 60 minutes at room temperature, and next incubated overnight with the primary antibody Setd8 (Q-18) sc: 54998 (Santa Cruz Biotechnology, Santa Cruz, CA) in 5% milk TBS-T at a concentration of 0.7μg/ml. After washing, they were incubated for an hour with a donkey anti-goat

secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 5% milk TBS-T. Membranes were washed again and exposed with enhanced chemiluminescence (ECL) according to the manufacturer's instructions (Amersham, Little Chalfont, UK) for 20 minutes to detect the signal.

After washing, membranes were stored overnight at 4°C. The next morning, prior to incubation with GAPDH in 5% milk TBS-T for 60 minutes at room temperature at a concentration of 0.1μg/ml the membranes were blocked a second time for 60 minutes in 5% milk TBS-T. Membranes were washed, incubated in anti-rabbit secondary antibody with 5% milk TBS-T for 60 minutes and exposed a second time according to specifics mentioned above. Identical procedures were followed for liver tissue samples with the following exceptions gel samples of 60μg total protein were used in place of 80μg and the membranes were blocked in 5% milk TBS-T overnight as opposed to 1 hour.

Statistics

Data are presented as means ± SEM. IUGR data were compared to control data that was normalized to 100%. Statistical significance between control and IUGR was determined using ANOVA. Statistical significance was defined as a P value ≤ 0.05.

RESULTS

IUGR Increases Setd8 Protein in VAT of Male Rats

Real-time RT-PCR was used to determine the abundance of Setd8 mRNA in VAT of IUGR versus control rats at day 21. IUGR did not have an effect on Setd8 mRNA abundance in VAT depots of male relative to the control (125±11% $P>0.05$). Also no significant differences were detected in mRNA abundance in VAT depots of IUGR females when compared to the control (85±6% $P>0.05$). See Figure 1.

Western blotting was used to determine the relative abundance of Sed8 protein in VAT of male and female IUGR versus control rats at day 21. IUGR increased Setd8 protein abundance in males relative to the controls (273±63% $P<0.05$). IUGR did not have a significant effect on Setd8 protein abundance of female relative to the controls (89±6% $P>0.05$). See Figure 2.

IUGR Has No Effect on Setd8 in SAT

Real-time RT-PCR was used to determine the relative abundance of Setd8 mRNA in SAT of male and female IUGR versus control rats at day 21. IUGR did not have an effect on Setd8 mRNA abundance in SAT depots of male relative to the control (115±7% $P>0.05$). Also no statistically significant differences were detected in mRNA abundance in SAT depots of IUGR females when compared to the control (90±9% $P>0.05$). See Figure 3.

Western blotting was used to determine the relative abundance of Sed8 protein in SAT tissue of male and female IUGR versus control rats at day 21. IUGR did not have an effect on Setd8 protein abundance in male relative to the controls (120±30% P>0.05). Also, IUGR did not have a significant effect on Setd8 protein abundance of female relative to the controls (102±23% P>0.05). See Figure 4.

IUGR Decreases Setd8 Protein in Liver of Female Rats

Real-time RT-PCR was used to determine the relative abundance of Setd8 mRNA in liver tissue of IUGR versus control rats at day 21. IUGR did not have an effect on Setd8 mRNA abundance in liver depots of male relative to the control (97±7% P>0.05). Also no statistically significant differences were detected in mRNA abundance in the liver of IUGR females when compared to the control (80±2% P>0.05). See Figure 5.

Western blotting was used to determine the relative abundance of Sed8 protein in liver tissue of male and female IUGR versus control rats at day 21. IUGR did not have a significant effect on Setd8 protein abundance in the liver of male relative to the controls (113±13% P>0.05). IUGR decreased Setd8 protein abundance in the liver of female relative to the controls (81±6% P<0.05). See Figure 6.

Gender Does Not Have an Effect on Setd8 mRNA

Real-time RT-PCR was used to determine the relative abundance of Setd8 mRNA in SAT, VAT and liver tissue of male control versus female control rats at day 21. There was no effect on Setd8 mRNA abundance in the SAT, VAT and liver tissue of male relative to the female. See Figure 7.

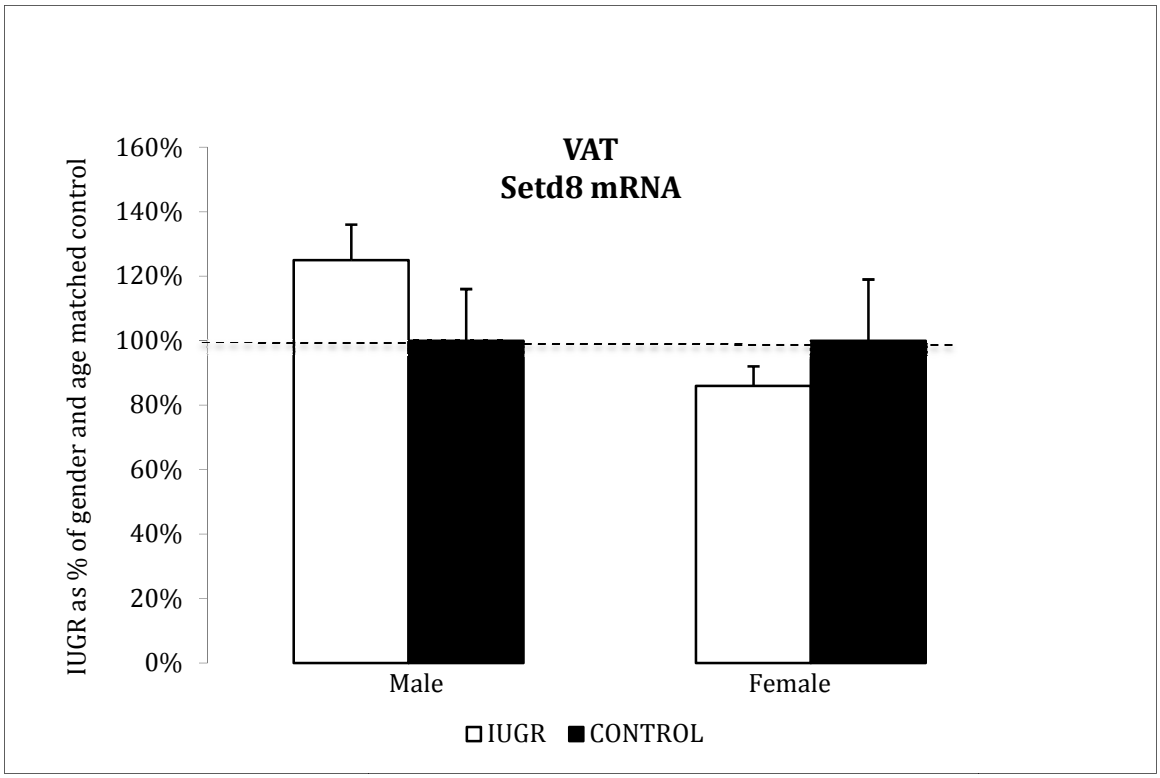

Figure 1. IUGR does not affect Setd8 mRNA abundance in VAT. The graph shows mRNA levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the controls. The dashed line represents control normalized to 100% and error bars are standard error of mean n=6.

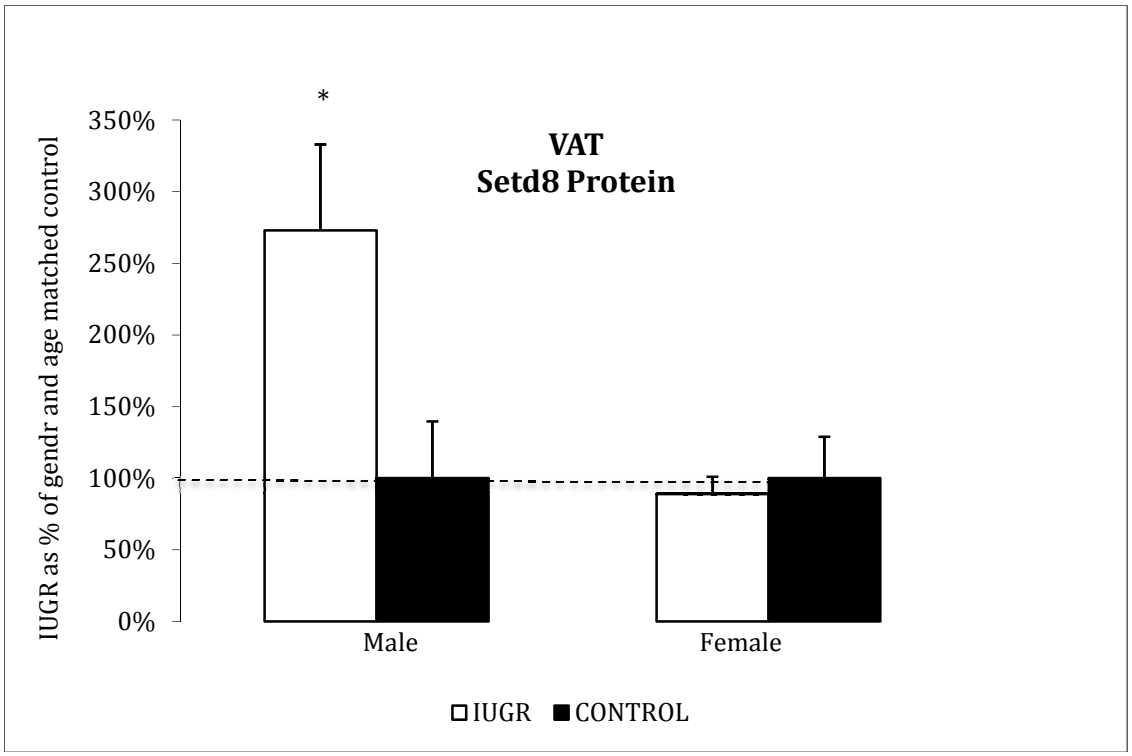

Figure 2. IUGR increases Setd8 protein abundance in male VAT. The graph shows protein levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the controls. The dashed line represents control normalized to 100% and error bars are standard error of mean n=6. *P<0.05.

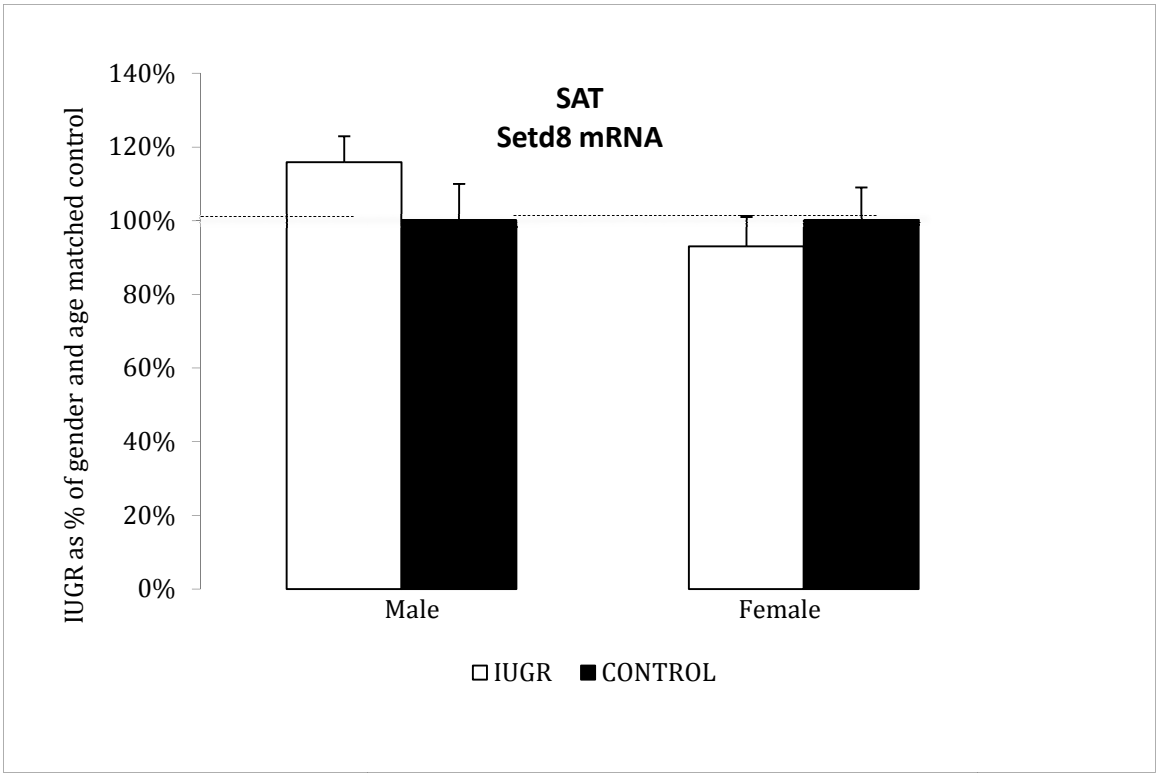

Figure 3. IUGR does not affect Setd8 mRNA abundance in SAT. The graph shows mRNA levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the controls. The dashed line represents controls normalized to 100% and error bars are standard error of mean n=6.

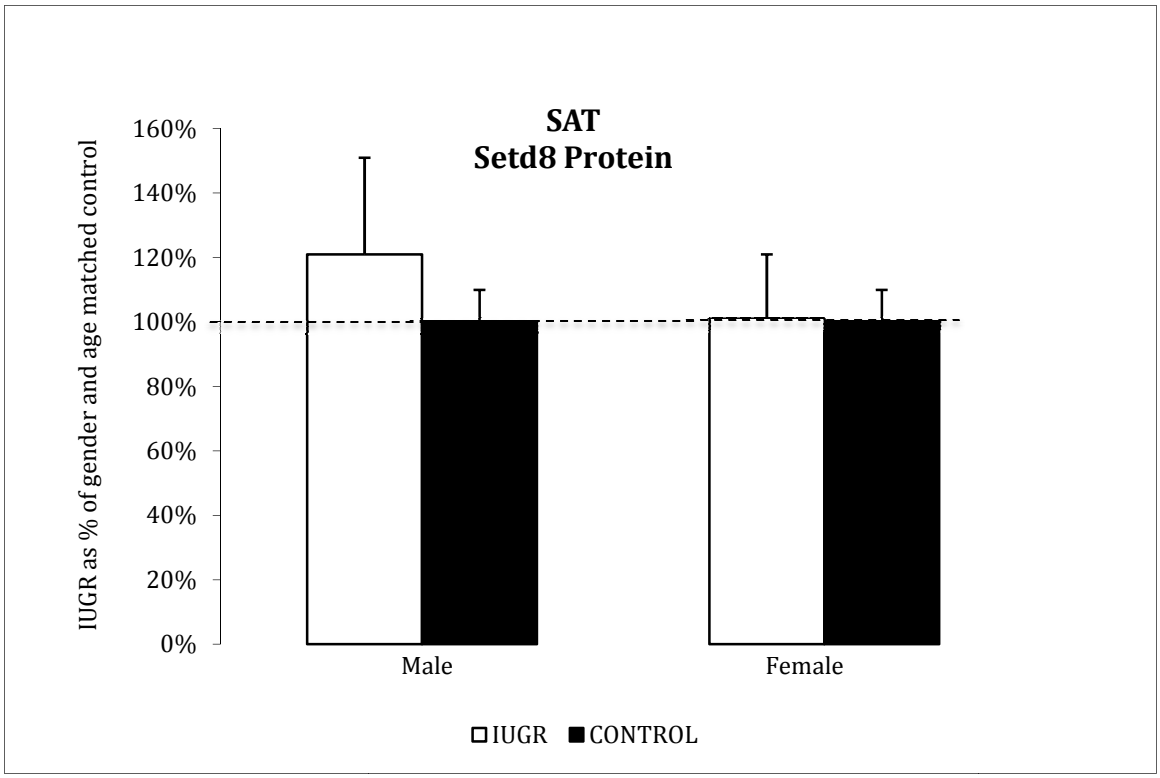

Figure 4. IUGR does not affect Setd8 protein abundance in the SAT. The graph shows protein levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the controls. The dashed line represents control normalized to 100% and error bars are standard error of mean n=6.

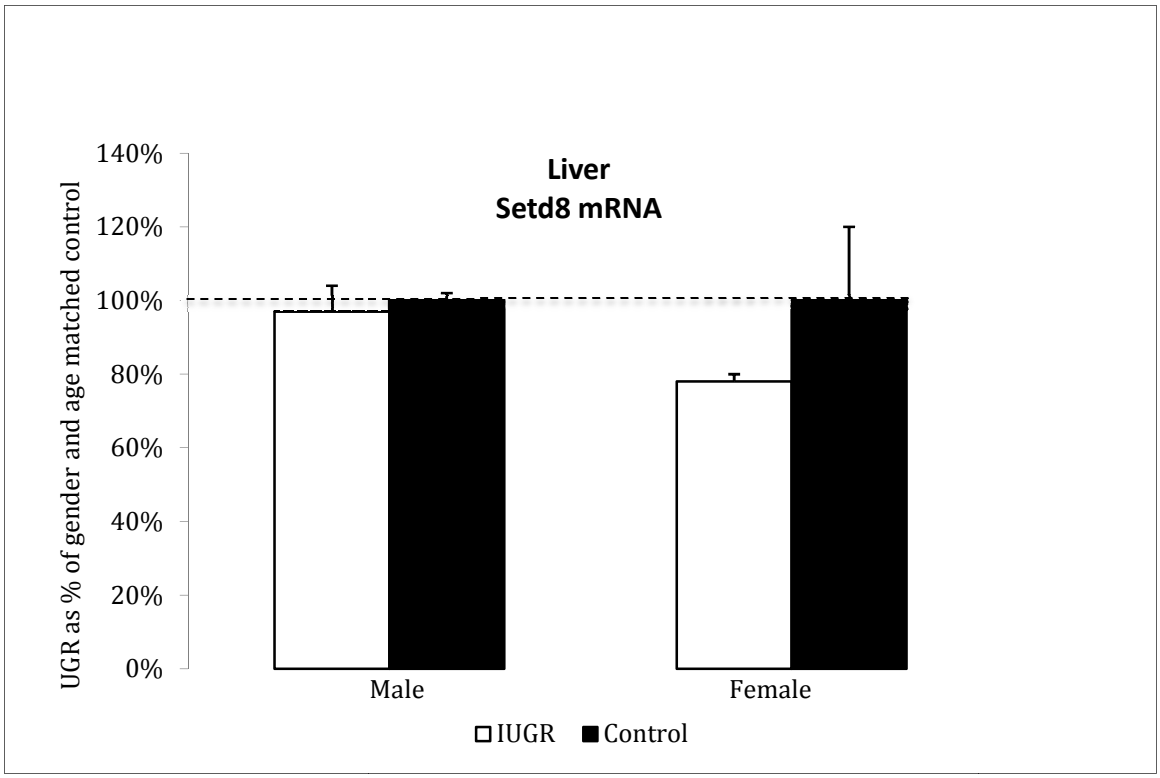

Figure 5. IUGR does not affect Setd8 mRNA abundance in liver. The graph shows mRNA levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the control. The dashed line represents control normalized to100% and error bars are standard error of mean n=6.

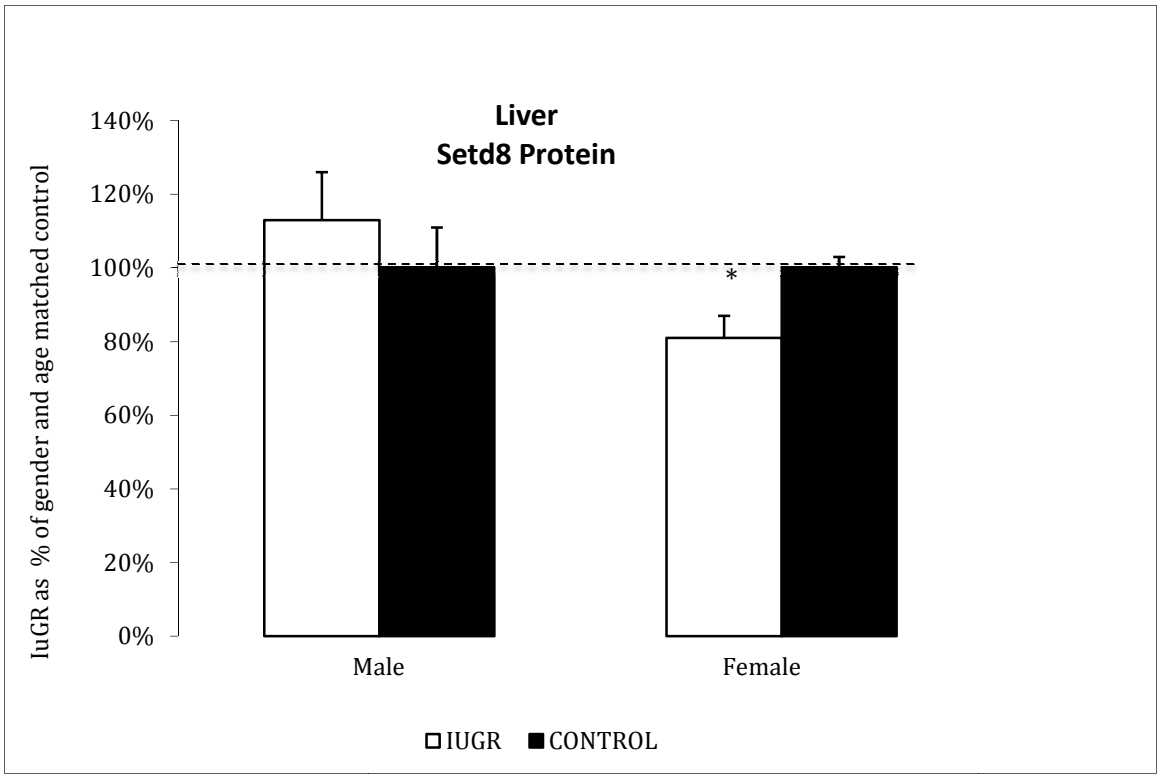

Figure 6. IUGR decreases Setd8 protein abundance in female liver. The graph shows protein levels of Setd8 expressed as a percent control. White solid bars represent IUGR and black solid bars represent the controls. The dashed line represents control normalized to 100% and error bars are standard error of mean n=6. *P<0.05.

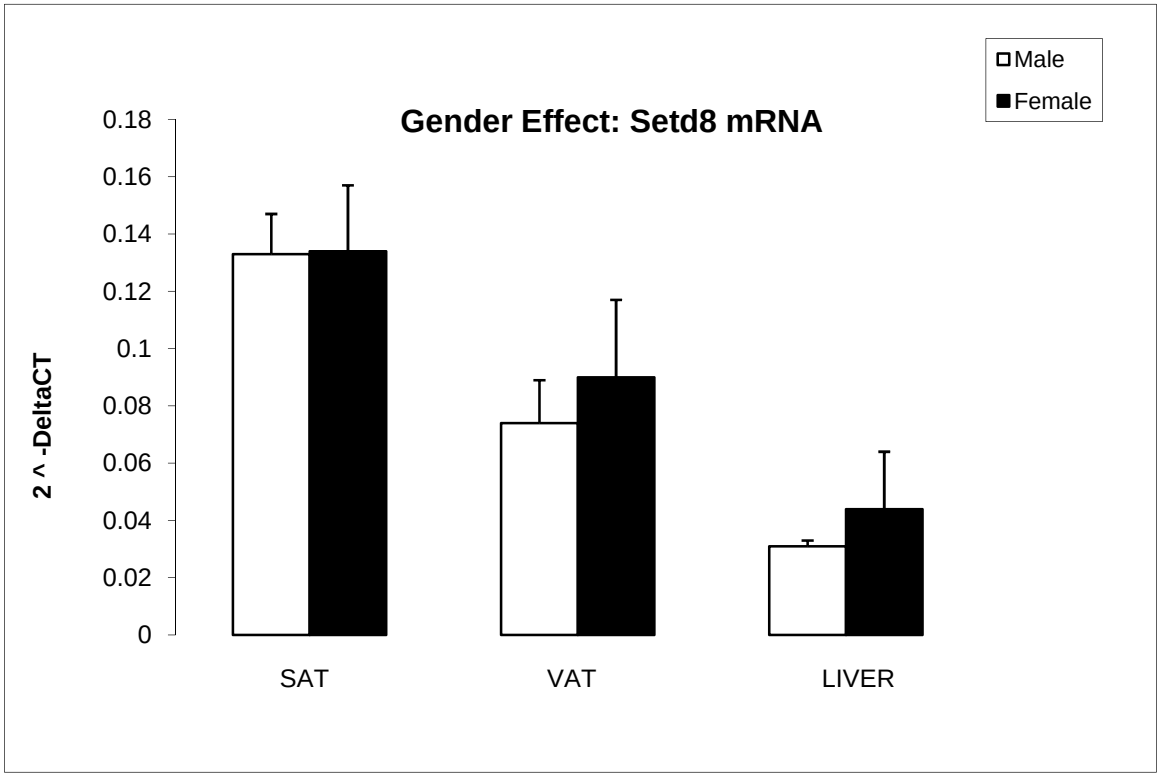

Figure 7. Gender does not affect Setd8 mRNA abundance in VAT, SAT or liver. The graph shows Detla CT values. White solid bars represent male and black solid bars represent the female. Error bars are standard error of mean n=6.

DISCUSSION

In this study we provide evidence that IUGR affects the expression of Setd8 in a gender and depot specific manner in VAT, SAT and hepatic tissue before the onset of obesity in postnatal day 21 rats. Evidence that IUGR affects expression of Setd8 in a gender and depot specific manner was demonstrated in VAT where IUGR increased the expression of Setd8 protein in male rat, and in liver where IUGR decreased the expression of Setd8 protein in females rat. Together these data suggest that Setd8 expression in IUGR is associated with the previously observed changes in PPARγ expression.

PPARγ regulation of the methyltransferase Setd8 has been recently documented (15) suggesting PPARγ's role in adipose and liver tissue has an epigenetic component. Epigenetic gene regulation refers to heritable changes in gene expression potential that are not caused by changes in DNA (25). One mechanism that contributes to epigenetic gene regulation is the methylation of histone tails. Setd8 puts a methyl mark in H4K20, which in turn increases the mark on genes, strongly correlating with an increase in gene expression. This IUGR-induced increase in Setd8 protein abundance in the VAT depots, and its association to increased levels of mRNA and protein of PPARγ, suggest a strong relationship to adipose tissue dysreguation in the male neonatal rat. Alternatively, in the liver of IUGR female rat the association of PPARγ and Setd8 decrease in protein expression may suggest that females are not as severely affected as males.

H4K20me1 at the exon 4 of the PPARγ gene during differentiation may determine PPARγ and other target gene expression. During differentiation PPARγ up regulates the expression of Setd8. Setd8 increases transcription of the PPARγ gene and its targets through H4K20me1. Further PPARγ binds to the PPARy2 promoter, inducing histone modification and ultimately increasing its transcription. Given our results, we can speculate that H4K20me1 is increased in the VAT of male IUGR and decreased in the liver tissue of female IUGR. This suggests that IUGR affects Setd8 and its targets in gender and tissue specific manner.

IUGR leads to adipose dysfunction, which favors the accumulation of VAT in conjunction with an increased expression of PPARγ before the onset of obesity (12). PPARγ can also influence adipose glucose uptake. PPARγ and retinoid x receptor (RXR) heterodimer repress transcriptional activity of the insulin responsive glucose transporter 4 (GLUT4) via direct and specific binding on its promoter (26). This transcriptional repression of GLUT4 is released via ligand binding on the PPARγ domain. In a recent study it has been observed that day 21 IUGR male rats have decreased insulin sensitive GLUT4 mRNA in VAT (12). We can also speculate that DHA supplementation of maternal diet may ameliorate adipose tissue regulation effecting PPARγ, Setd8, and H4K20me1 levels of Setd8 and its targets. This benefit of a DHA maternal diet has already been shown in the IUGR lung (27). It is possible that the feedback mechanism that links PPARγ transcription and epigenetic gene regulation through Setd8 H4K20me1 may provide a link into the IUGR, and its effect on insulin resistance.

In conclusion we have demonstrated in a rat IUGR model that Setd8 expression varies in SAT, VAT and hepatic tissue in a gender and depot specific manner. PPARγ and

its target histone modifying enzyme Setd8 expression seem to be strongly related to H4K20me1 levels on the PPARγ gene. Further H4K20me1 modifications on Setd8 targets in a genders and tissue specific manner suggests IUGR alters epigenetic gene regulation,

and ultimately the phenotype before the adult onset of metabolic disease and obesity.

Potential Impact

The importance of intrauterine environment for lifelong health and disease has been studied extensively. The adaption of the fetus to IUGR leads to permanent changes in metabolism, body composition and growth. The adjustment to a poor and stressful intrauterine environment has a programming effect on lipid and glucose metabolism and chronic disease risk. The IUGR infants have physiologically impaired adipose tissue regulation, which in adulthood is accompanied by adult onset metabolic disease, insulin resistance, poor fatty acid utilization, hepatic steatosis and obesity. Given the prevalence and impact of such diseases in developed nations we aimed to shed some light in problem and looked at novel epigenetic mechanisms. This research project provides the first in vivo comprehensive characterization in adipose and liver tissue of how IUGR affects the production of the chromatin modifying enzyme Setd8 in a gender and tissue specific manner. This is important because it allows us to get a step closer to the development of interventions that may improve the IUGR phenotype.

Strength and Limitations

In order to establish the effect of the perinatal environment on the epigenetic modulation of transcription an in vivo model is necessary. The IUGR model provides us

with the best current system of understanding on how epigenetics modulates gene transcription in vivo. However, this model may not be an exact representative of the human condition. Our study describes how IUGR effects Setd8 in a gender and tissue specific manner, however more studies are needed to explore the mechanism by which these specificity is attained. For this study we looked at only one age, but the gender differences may be more prominent as the animals develop.

REFERENCES

1. Hoyert DL, Mathews TJ, Menacker F, Strobino DM, Guyer B. Annual summary of vital statistics: 2004. Pediatrics 2006;117(1):168-83. doi: 10.1542/peds.2005-2587.

2. Kinzler WL, Vintzileos AM. Fetal growth restriction: a modern approach. Curr Opin Obstet Gynecol 2008;20(2):125-31. doi: 10.1097/GCO.0b013e3282f7320a.

3. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet 2005;365(9461):785-99. doi: 10.1016/S0140-6736(05)17987-2.

4. Cetin I, Alvino G. Intrauterine growth restriction: implications for placental metabolism and transport. A review. Placenta 2009;30 Suppl A:S77-82. doi: 10.1016/j.placenta.2008.12.006.

5. Rosenberg A. The IUGR newborn. Semin Perinatol 2008;32(3):219-24. doi: 10.1053/j.semperi.2007.11.003.

6. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr 2007;27:363-88. doi: 10.1146/annurev.nutr.27.061406.093705.

7. Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. Diabetes 2001;50(10):2279-86.

8. Muhlhausler B, Smith SR. Early-life origins of metabolic dysfunction: role of the adipocyte. Trends Endocrinol Metab 2009;20(2):51-7. doi: 10.1016/j.tem.2008.10.006.

9. Parekh S, Anania FA. Abnormal lipid and glucose metabolism in obesity: implications for nonalcoholic fatty liver disease. Gastroenterology 2007;132(6):2191-207. doi: 10.1053/j.gastro.2007.03.055.

10. Ibanez L, Lopez-Bermejo A, Suarez L, Marcos MV, Diaz M, de Zegher F. Visceral adiposity without overweight in children born small for gestational age. J Clin Endocrinol Metab 2008;93(6):2079-83. doi: 10.1210/jc.2007-2850.

11. Yajnik CS, Lubree HG, Rege SS, et al. Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 2002;87(12):5575-80.

12. Joss-Moore LA, Wang Y, Campbell MS, et al. Uteroplacental insufficiency increases visceral adiposity and visceral adipose PPARgamma2 expression in male rat offspring prior to the onset of obesity. Early Hum Dev 2010;86(3):179-85. doi: 10.1016/j.earlhumdev.2010.02.006.

13. Bispham J, Gardner DS, Gnanalingham MG, Stephenson T, Symonds ME, Budge H. Maternal nutritional programming of fetal adipose tissue development: differential effects on messenger ribonucleic acid abundance for uncoupling proteins and peroxisome proliferator-activated and prolactin receptors. Endocrinology 2005;146(9):3943-9. doi: 10.1210/en.2005-0246.

14. Muhlhausler BS, Duffield JA, McMillen IC. Increased maternal nutrition stimulates peroxisome proliferator activated receptor-gamma, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. Endocrinology 2007;148(2):878-85. doi: 10.1210/en.2006-1115.

15. Wakabayashi K, Okamura M, Tsutsumi S, et al. The peroxisome proliferator-activated receptor gamma/retinoid X receptor alpha heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a positive feedback loop. Mol Cell Biol 2009;29(13):3544-55. doi: 10.1128/MCB.01856-08.

16. Zhu Y, Qi C, Korenberg JR, et al. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. Proc Natl Acad Sci U S A 1995;92(17):7921-5.

17. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444(7121):847-53. doi: 10.1038/nature05483.

18. Desai M, Guang H, Ferelli M, Kallichanda N, Lane RH. Programmed upregulation of adipogenic transcription factors in intrauterine growth-restricted offspring. Reprod Sci 2008;15(8):785-96. doi: 10.1177/1933719108318597.

19. Jenuwein T, Allis CD. Translating the histone code. Science 2001;293(5532):1074-80. doi: 10.1126/science.1063127.

20. Fang J, Feng Q, Ketel CS, et al. Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. Curr Biol 2002;12(13):1086-99.

21. Wu S, Wang W, Kong X, et al. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes Dev 2010;24(22):2531-42. doi: 10.1101/gad.1984210.

22. Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol 2002;283(2):R281-3. doi: 10.1152/ajpregu.00279.2002.

23. Kloesz JL, Serdikoff CM, Maclennan NK, Adibi SA, Lane RH. Uteroplacental insufficiency alters liver and skeletal muscle branched-chain amino acid metabolism in intrauterine growth-restricted fetal rats. Pediatr Res 2001;50(5):604-10.

24. Ogata ES, Bussey ME, LaBarbera A, Finley S. Altered growth, hypoglycemia, hypoalaninemia, and ketonemia in the young rat: postnatal consequences of intrauterine growth retardation. Pediatr Res 1985;19(1):32-7.

25. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33 Suppl:245-54. doi: 10.1038/ng1089.

26. Armoni M, Kritz N, Harel C, et al. Peroxisome proliferator-activated receptor-gamma represses GLUT4 promoter activity in primary adipocytes, and rosiglitazone alleviates this effect. J Biol Chem 2003;278(33):30614-23. doi: 10.1074/jbc.M304654200.

27. Joss-Moore LA, Wang Y, Baack ML, et al. IUGR decreases PPARgamma and SETD8 Expression in neonatal rat lung and these effects are ameliorated by maternal DHA supplementation. Early Hum Dev 2010;86(12):785-91. doi: 10.1016/j.earlhumdev.2010.08.026.